Metabolic Fingerprinting of Sabah *Ruellia tuberosa* Plant Extracts for the Identification of Potential Anticancer Compounds

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ABSTRACT

The aim of this study was to identify the potential anticancer compounds of the Sabah Ruellia tuberosa through metabolic fingerprinting approach. In this study, gas chromatography-mass spectrometry (GC-MS) was used to obtain the fingerprints of Ruellia tuberosa plant extracts. GC-MS analysis of R. tuberosa leaf extracts revealed the presence of 15 compounds; squalene, vitamin E, campesterol, stigmasterol, gamma-tocopherol, gamma-sitosterol, 9, 12, 15-octadecatrienoic acid (Z, Z, Z), alpha-amyrin, hexadecanoic acid trimethylsilyl ester, cholesterol, alpha-linolenic acid trimethylsilyl ester, sucrose, octakis (trimethylsilyl) ether, alpha-tocopherol trimethylsilyl ether, silane, [[(3ϐ, 24R)-ergost-5-en-3-yl]oxy]trimethyl- and beta-sitosterol trimethylsilyl ether. Meanwhile, 10 compounds were detected in the stem extracts; vitamin E, squalene, stigmasterol, campesterol, gamma-sitosterol, lupeol, sucrose, octakis (trimethylsilyl) ether, silane, [[3ϐ, 24R)-ergost-5-en-3-yl]oxy]trimethyl-, beta-sitosterol trimethylsilyl ether and alpha-tocopherol trimethylsilyl ether. Similarly, 10 compounds were detected in root extracts; vitamin E, stigmasterol, campesterol, squalene, gamma-sitosterol, lupeol, alpha-tocopherol trimethylsilyl ether, hexadecanoic acid trimethylsilyl ester, silane, [[3ϐ, 24R)-ergost-5-en-3-yl] oxy]trimethyl- and beta-sitosterol trimethylsilyl ether. A chemical database was constructed using PCDL software. By analysing the chemical database and the MTT assay of R. tuberosa against breast cancer (MCF-7) cell line, the potential anticancer compounds were speculated. We highly speculated that squalene, stigmasterol, campesterol, vitamin E and lupeol were the compounds responsible for the anticancer activity of Ruellia tuberosa. Therefore, further study should be conducted to isolate these compounds and study their proliferative activity against MCF-7 cell line.

Keywords: *Ruellia tuberosa, Anticancer, Metabolic fingerprinting, GC-MS*

INTRODUCTION

Cancer can be defined as the uncontrolled growth of abnormal cells in the body and is considered as one of the leading causes of death worldwide. Conventional cancer therapies may cause serious side effects and at best, only extend patient's lifetime by a few year (Amin *et al.*, 2009). Hence, alternative approaches were considered. Plants have been used traditionally for medicinal purposes. The importance of plants lies not only in their therapeutic value but also in their potential as sources of novel drug (Vital and Rivera, 2011). Natural compounds from the plants are being used as anticancer as well as chemopreventive compounds (Amin *et al.*, 2009). Plants were recognized as the source of anticancer in the 1950s with the discovery and development of vinca alkaloids and isolation of cytotoxic podohyllotoxins (Bhanot *et al.*, 2011).

Ruellia tuberosa Linn. is a Minnie root plant; a tropical perennial plant that is widely distributed in Southeast Asia (Chen *et al.*, 2006; Arirudran *et al.*, 2011b). It belongs to family Acanthaceae and has been used medicinally in West Indies, Central America, Guiana and Peru (Chothani and Mishra, 2012) as an anthelmintic, against joint pains, strained muscles, diuretic, antidiabetic, antipyretic, analgesic, anti-hypertensive and antidotal agent (Arirudran *et al.*, 2011a). Recently, it has been incorporated as one of the components in an herbal drink in Taiwan (Chen *et al.*, 2006). Various experiments have been conducted and it is proven that *R. tuberosa* possess biological activities such as antioxidant (Chen *et al.*, 2006; Chothani and Mishra, 2012; Geetha *et al.*, 2013), antimicrobial (Arirudran *et al.*, 2011a), anticancer (Reddy *et al.*, 2013) antihyperlipidemic (Krishna *et al.*, 2012), gastroprotective activity (Arambewela *et al.*, 2003), antidiabetic and hepatoprotective activity (Rajan *et al.*, 2012). Recently, Cheong *et al.* (2013) have studied about the *R. tuberosa* from Sabah and found that the plants possess excellent anticancer activity especially against the breast cancer (MCF-7) cell line.

Despite its potential value, there is relatively little information on its chemical constituent and pharmacological activities on *R. tuberosa* (Arirudran *et al.*, 2011a; Chen *et al.*, 2006). The knowledge of the chemical constituents of plants is necessary for the discovery of potential therapeutic agents (Sermakkani and Thangapandian, 2012). Hence, the aim of this study is to obtain the metabolic fingerprints of Sabah *R. tuberosa* plant extracts using gas chromatography-mass spectrometry (GC-MS) approach. From the GC-MS analysis, a database/library was constructed using the PCDL software. Hence, the results from this study might give us further insight on the chemical constituents of *R. tuberosa*, especially in those found in Sabah, to be used to identify the potential anticancer compounds of this plant.

MATERIALS AND METHODS

Chemicals and reagents

The solvents used for extraction and GC-MS analysis were hexane, chloroform, ethyl acetate and methanol and were obtained from Fischer Scientific (Pitsburgh, PA, USA). Solvents used for extraction were of analytical grade while the solvents used for GC-MS analysis were of HPLC grade. Apart from that, both the derivatization reagents, N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were purchased from Sigma Aldrich (USA).

Plant materials

Fresh plant materials were collected in August 2013 at the side drain of Ujana Kingfisher Park, Kota Kinabalu. The plants were identified by a botanist at Universiti Malaysia Sabah. The specimen voucher (ACRT01/2013) was deposited at the herbarium of the university. The whole plants were washed and allowed to dry at room temperature. The plants were then divided into three part; leaf, stem and root. The dried plants (leaf, stem, root) were pulverized using heavy duty blender and stored in -80°C for further analysis.

Preparation of methanolic extract of *Ruellia tuberosa* and its fractions

The methanolic extract (ME) was prepared according to Cheong *et al.* (2013). Approximately 12 g of plant powder was subjected to extraction using the Soxhlet extractor with 300 ml of absolute methanol. The ME was then evaporated to obtain viscous semi sold masses. The ME was further fractionated to obtain different fractions through solvent-solvent partitioning as described by Chen *et al.* (2006). Solvents used were hexane, chloroform, ethyl acetate and water. The ME and its four fractions of hexane (HxF), chloroform (CfF), ethyl acetate (EaF) and water (WtF) were evaporated and stored in -80°C for further analysis.

Derivatisation of methanolic extract of *Ruellia tuberosa*

The methanolic extracts (ME) were subjected to derivatization to reduce the polarities of the functional group as well as to facilitate separation by GC-MS. The silylation procedure was done as according to Proetos *et al.* (2006) with slight modifications. A mixture of TMCS (50 µl) and BSTFA (650 µl) were added into 2 ml vial containing the ME (leaf, stem and root). The mixture was vortexed and subsequently sonicated for 45 min. From the silylated mixture, 1 µl was injected onto the GC-MS column.

GC-MS analysis

GC-MS analysis was performed with an Agilent 7890A series gas chromatograph equipped with a 7693 autosampler, an HP-5MS column (30 m X 0.250 mm i.d.,

film thickness of 0.25 micron) and a 5975C mass spectrometer with triple axis detector. The parameters of GC-MS were set as described by Fischedick *et al.* (2010) with slight modifications. The MS source was set to 230°C , the MS quad temperature was 150°C and the transfer line temperature was set to 280°C . The injector temperature was 250°C with an injection volume of 1 µl sample in a splitless mode and a carrier gas (Helium) with the flow rate of 1 ml/min. The oven temperature was programmed from 220°C for 10 min then increased at a rate of 5°C /min until it reached 300°C and held for 15 min. The post run was set at 200°C for 5 min. Ion source temperature was maintained at 200°C . The mass spectrum of compounds in the sample extracts was obtained by electron ionization at 70 eV. The total running time of GC-MS analysis was 41 min. A 1 µl aliquot of each sample extracts was injected onto the GC column. Each extract was injected twice. Identification of sample was done by comparison with the National Institute of Standards and Technology (NIST) database. The name, molecular weight, molecular formula and structure of the compounds were ascertained.

Database/library construction and identification of potential anticancer compounds

A database/library for the chemical constituents of *R. tuberosa* was constructed using the MassHunter Personal Compound Database and Library Manager (PCDL) software (Agilent, USA). The information related to the detected compounds were entered into the created PCDL including the name, mass, RT, molecular formula, CAS or ChemSpider ID. Based on the constructed database, the identity/abundance of each of the compound present in each of the extract with its anticancer activity was compared and analysed. Finally, the potential anticancer compounds were postulated and were set as a target for isolation and anticancer study in the future.

RESULTS AND DISCUSSION

GC-MS analysis of leaves extracts of *R. tuberosa* revealed the presence of 15 compounds. The compounds, their retention time (RT), molecular formula, molecular weight (MW) and peak area were summarised in Table 1. GC-MS analysis of methanolic extract (ME) identified 3 compounds; squalene (17.65%), vitamin E (14.68%) and stigmasterol (6.09%). For hexane extract (HxF), GC-MS revealed a total of 6 compounds with squalene (23.67%) as the major constituent, followed by vitamin E (17.03%), stigmasterol (5.25%), gamma-sitosterol (2.87%), campesterol (2.77%) and gamma-tocopherol (1.04%). Meanwhile, GC-MS of chloroform extract (CfF) revealed the presence of 9 compounds such as 9, 12, 15-octadecanoic acid (Z, Z, Z) (38.66%), squalene (12.38%), vitamin E (11.84%),

stigmasterol (6.28%), gamma-sitosterol (3.50%), campesterol (2.68%), alpha-amyrin (1.38%), cholesterol (1.02%) and gamma-tocopherol (0.80%). Only 1 compound detected by GC-MS of ethyl acetate extract (EaF); squalene (74.74%) while 8 compounds detected in GC-MS of derivatized methanol (DME) with squalene (23.57%) as the major constituent, followed by alpha-tocopherol trimethylsilyl ether (22.37%), stigmasterol trimethylsilyl ether (8.64%), alpha-linolenic acid trimethylsilyl ester (7.96%), hexadecanoic acid trimethylsilyl ester (5.83%), beta-sitosterol trimethylsilyl ether (4.69%), silane, [[(3β,24R)-ergost-5-en-3-yl]oxy]trimethyl- (3.28%) and sucrose, octakis (trimethylsilyl) ether (3.16%).

Table 1 GC-MS analysis revealed the presence of phytochemical compounds in various leaves extracts of *Ruellia tuberosa*

Extracts	RT (min)	Compounds	Molecular Formula	MW	% Area
ME	18.753	Squalene	$C_{30}H_{50}$	410.391	17.65
	23.322	Vitamin E	$C_{29}H_{50}O_2$	430.381	14.68
	24.983	Stigmasterol	$C_{29}H_{48}O$	412.371	6.09
HxF	18.881	Squalene	$C_{30}H_{50}$	410.391	23.67
	22.299	Gamma-Tocopherol	$C_{28}H_{48}O_2$	416.365	1.04
	23.503	Vitamin E	$C_{29}H_{50}O_2$	430.381	17.03
	24.631	Campesterol	$C_{28}H_{48}O$	400.371	2.77
	25.079	Stigmasterol	$C_{29}H_{48}O$	412.371	5.25
	25.835	Gamma-Sitosterol	$C_{29}H_{50}O$	414.386	2.87
CfF	4.802	9,12,15-Octadecatrienoic acid (Z,Z,Z)	$C_{18}H_{30}O_2$	278.225	38.66
	18.785	Squalene	$C_{18}H_{30}O_2$	410.391	12.38
	22.214	Gamma-Tocopherol	$C_{28}H_{48}O_2$	416.365	0.80
	23.045	Cholesterol	$C_{27}H_{46}O$	386.355	1.02
	23.375	Vitamin E	$C_{29}H_{50}O_2$	430.381	11.84
	24.525	Campesterol	$C_{28}H_{48}O$	400.371	2.61
	24.983	Stigmasterol	$C_{29}H_{48}O$	412.371	6.28
	25.760	Gamma-Sitosterol	$C_{29}H_{50}O$	414.386	3.50
	26.708	Alpha-Amyrin	$C_{30}H_{50}O$	426.386	1.38
EaF	18.753	Squalene	$C_{18}H_{30}O_2$	410.391	74.74
DME	3.272	Hexadecanoic acid trimethylsilyl ester	$C_{19}H_{40}O_2Si$	326.279	5.83
	5.728	Alpha-Linolenic acid trimethylsilyl ester	$C_{21}H_{38}O_2Si$	350.611	7.96
	16.623	Sucrose, octakis (trimethylsilyl) ether	$C_{36}H_{86}O_{11}Si_8$	918.432	3.16
	18.753	Squalene	$C_{18}H_{30}O_2$	410.391	23.57
	23.620	Alpha-Tocopherol trimethylsilyl ether	$C_{32}H_{58}O_2Si$	502.421	22.37
	25.047	Silane, [[(3β,24R)-ergost-5-en-3-yl]oxy]trimethyl-	$C_{31}H_{56}OSi$	472.410	3.28
	25.473	Stigmasterol trimethylsilyl ether	$C_{32}H_{56}OSi$	484.410	8.64
	26.197	Beta-Sitosterol trimethylsilyl ether	$C_{32}H_{58}OSi$	486.426	4.69

GC-MS analysis of stem extracts of *R. tuberosa* revealed a total of 10 compounds. Table 2 presented the RT, molecular formula, MW and peak area (%) of those compounds. From the table, GC-MS of ME revealed the presence of 2 compounds; lupeol (35.43%) and vitamin E (2.97%). GC-MS of HxF revealed the presence of 6 compounds with lupeol (47.62%) as the major constituent followed by stigmasterol (8.71%), gamma-sitosterol (5.40%), vitamin E (5.19%), campesterol (3.29%) and squalene (2.66%). GC-MS of CfF revealed the presence of 5 compounds; lupeol (24.35%), stigmasterol (3.98%), gamma-sitosterol (2.68%), vitamin E (1.96%) and campesterol (1.70%) while only 1 compound detected in EaF which was squalene (88.19%). Meanwhile 5 compounds detected by GC-MS of DME; sucrose, octakis (trimethylsilyl) ether (68.07%), stigmasterol trimethylsilyl ether (2.19%), beta-

sitosterol trimethylsilyl ether (1.88%), alpha-tocopherol trimethylsilyl ether (1.79%) and silane, [[(3β, 24R)-ergost-5-en-3-yl] oxy]trimethyl- (1.10%).

Table 2 GC-MS analysis revealed the presence of phytochemical compounds in various stems extracts of *Ruellia tuberosa*

Extracts	RT	Compounds	% Area
ME	23.396	Vitamin E	2.97
	26.761	Lupeol	35.43
HxF	18.785	Squalene	2.66
	23.417	Vitamin E	5.19
	24.631	Campesterol	3.29
	25.025	Stigmasterol	8.71
	25.835	Gamma-Sitosterol	5.40
	26.900	Lupeol	47.62
CfF	23.375	Vitamin E	1.96
	24.578	Campesterol	1.70
	25.004	Stigmasterol	3.98
	25.760	Gamma-Sitosterol	2.68
	26.774	Lupeol	24.35
EaF	18.732	Squalene	88.19
DME	16.676	Sucrose, octakis (trimethylsilyl) ether	68.07
	23.577	Alpha-Tocopherol trimethylsilyl ether	1.79
	25.057	Silane, [[(3β,24R)-ergost-5-en-3-yl]oxy]trimethyl-	1.10
	25.451	Stigmasterol trimethylsilyl ether	2.19
	26.208	Beta-Sitosterol trimethylsilyl ether	1.88

Similarly, GC-MS analysis of root extracts revealed the presence of 10 compounds as summarised in Table 3. GC-MS of ME revealed the presence 3 compounds such as lupeol (73.84%), stigmasterol (3.96%) and vitamin E (1.68%) while GC-MS of HxF revealed 5 compounds; lupeol (66.32%), stigmasterol (7.19%), gamma-sitosterol (2.96%), campesterol (2.18%) and vitamin E (1.99%). Only 1 compound detected in GC-MS of CfF and EaF which was lupeol (23.10%) and squalene (86.61%), respectively. Meanwhile, 5 compounds were detected in GC-MS of DME stigmasterol trimethylsilyl ether (2.39%), hexadecanoic acid trimethylsilyl ester (1.63%), silane, [[(3β, 24R)-ergost-5-en-3-yl]oxy] trimethyl- (1.15%), beta-sitosterol trimethylsilyl ether (1.06%) and alpha-tocopherol trimethylsilyl ether (1.05%).

Table 3 GC-MS analysis revealed the presence of phytochemical compounds in various roots extracts of *R. tuberosa*

Extracts	RT	Compounds	% Area
ME	23.364	Vitamin E	1.68
	24.983	Stigmasterol	3.96
	26.761	Lupeol	73.84
HxF	23.396	Vitamin E	1.99
	24.706	Campesterol	2.18
	25.079	Stigmasterol	7.19
	25.835	Gamma-Sitosterol	2.96
	26.968	Lupeol	66.32
CfF	26.789	Lupeol	23.10
EaF	18.732	Squalene	86.61
DME	3.645	Hexadecanoic acid, trimethylsilyl ester	1.63
	23.577	Alpha-Tocopherol trimethylsilyl ether	1.05
	25.057	Silane, [[(3β, 24R)-ergost-5-en-3-yl] oxy] trimethyl-	1.15
	25.451	Stigmasterol trimethylsilyl ether	2.39
	26.186	Beta-Sitosterol trimethylsilyl ether	1.06

By interpreting the compounds detected in leaf, stem and root extracts of *R. tuberosa*, we found that this plant possesses various pharmacological value. The pharmacological activity of these compounds is presented in Table 4. These activities were agreeable with the finding from Cheong *et al.* (2013) and Reddy *et al.* (2013) in which they reported the anticancer property of *R. tuberosa* against MCF-7 cell line and breast tumour induce by Ehrlich Ascites Carcinoma (EAC) respectively. Furthermore, the antidiabetic activity as well as hypocholesterolemic of some of the compounds (squalene, gamma-sitosterol) were in correlation with the significant blood glucose lowering effect as reported by Shahwar *et al.* (2011) in normal and alloxan-induced diabetic rabbits and reduction in cholesterol and triglycerides level of diabetic rat (Ananthakrishnan and Doss, 2012). Among all, the most prevalent phytochemical compounds found in Sabah *R. tuberosa* plant extracts were squalene, vitamin E, stigmasterol and campesterol. Another compound that present in high amount was lupeol, which was detected in stem and root extracts of *R. tuberosa*. Their mass spectrum is shown in Figure 1.

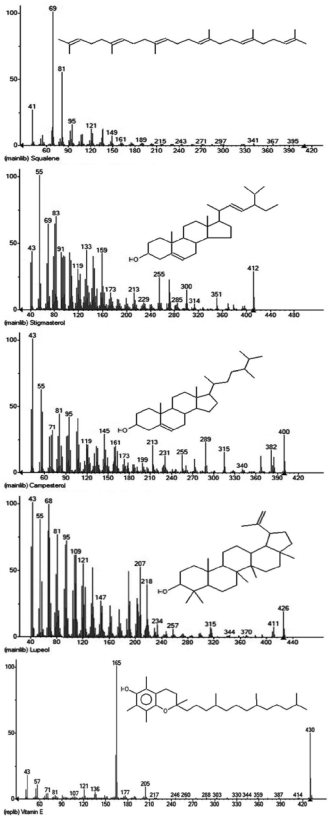

Figure 1 The mass spectrums for prevalent compounds in *R. tuberosa* plant extracts; a) Squalene; b) Stigmasterol; c) Campesterol; d) Lupeol; & e) Vitamin E

Squalene is a triterpene which is an intermediate of the cholesterol biosynthesis pathway (Huang *et al.*, 2009). It is indicated that this compound was present in almost all the extracts particularly in ME, HxF, CfF and EaF of *R. tuberosa* leaf and stem, while present in HxF and EaF of *R. tuberosa* root. Squalene is a highly unsaturated aliphatic hydrocarbon which belongs to the triterpene groups of oil (Wejnerowska *et al.*, 2013). It is a biochemical precursor of cholesterol and other steroids (Reddy and Couvreur, 2009), thus is one of the reasons why it can be detected in almost all of the *R. tuberosa* extracts. Apart from that, squalene is widespread in nature and consumed as an integral part of the human diet in addition to being synthesized within cells (Reddy and Couvreur, 2009). These authors also reported squalene as a potential chemopreventive agent, especially for breast, pancreatic and colon carcinomas.

Another predominant compound detected in *R. tuberosa* plant extracts was vitamin E. The function of vitamin E as an antioxidant to human health is widely known. Vitamin E functions as lipid-soluble antioxidants by scavenging lipid peroxyl radicals generated in the cellular membranes (Ulatowski *et al.*, 2014). Additionally, vitamin E functions as anti-inflammatory, antimicrobial and anti-spasmodic (Venkata *et al.*, 2012). Apart from vitamin E, there are four tocopherols and four tocotrienols with alpha (α), beta (β), gamma (γ) and delta (δ) isomers. Among these, gamma-tocopherol was found to be present in *R. tuberosa* plant extracts. This compound acts as antioxidants, anti-inflammatory and anticancer (Jiang *et al.*, 2004). The properties of these two compounds (vitamin E and gamma-tocopherol) could be responsible for the antioxidant (Chen *et al.*, 2006) and antimicrobial (Arirudran *et al.*, 2011a) properties of *R. tuberosa*.

Table 4 Biological activities of phytochemical compounds detected in leaves, stems and root extracts of *Ruellia tuberosa*

Name of Compounds	Activities	Reference
Squalene	Antibacterial, antioxidant, pesticide, antitumor, cancer preventive, immunostimulant, chemopreventive, lipoxygenase-inhibitor	Sermakkani & Thangapandian, 2012
Vitamin E	Antioxidant, anti-inflammatory, antimicrobial, radical scavenging, anti-spasmodic	Venkata *et al*., 2012
Stigmasterol	Antimicrobial, anticancer, anti-inflammatory, anti-asthma, diuretic, antiarthritic	Rajendra *et al*., 2014
Gamma-Tocopherol	Antioxidant, anti-inflammatory, anticancer	Hensley *et al*., 2004; Jiang *et al*., 2004
Campesterol	Antinociceptive activity, anticancer	Kamurthy *et al*., 2013; Choi *et al*., 2007
Gamma-Sitosterol	Antifungal, antibacterial, anti-angiogenic activity, antidiabetic	Venkata *et al*., 2012
9,12,15-Octadecatrienoic acid (Z,Z,Z)	Anti-inflammatory, nematicide, insectifuge, hypocholesterolomic, cancer preventive, hepatoprotective, antihistamic, antiacne, antiarthritic, antieczemic	Sermakkani & Thangapandian, 2012
Lupeol	Antimicrobial, anticancer, anti-inflammatory, antioxidant, antiarthritic	Rajendra *et al*., 2014
Alpha-Amyrin	Antiedemic, anti-inflammatory, antitumor, hepatoprotective, insectifuge, anti-hyperglycemic activity.	Devi *et al*., 2011
Hexadecanoic acid trimethylsilyl ester	Antioxidant, nematicide, hypocholesterolemic, pesticide	Jain *et al*., 2012
Alpha-Linolenic acid trimethylsilyl ester	Free radical scavenger, antidepressant effect, anti-inflammatory, hypocholesterolemic	Zhou *et al*., 2004; Blondeau *et al*., 2009
Alpha-Tocopherol trimethylsilyl ether	Antioxidant	Yang *et al*., 2007
Stigmasterol trimethylsilyl ether	Antimicrobial, anticancer, anti-inflammatory, anti-asthma, diuretic, antiarthritic	Rajendra *et al*., 2014
Beta-Sitosterol trimethylsilyl ether	Antimicrobial, anti-inflammatory, analgesic, anticancer, antiasthma, diuretic, antiarthritic	Jain *et al*., 2012; Rajendra *et al*., 2014

Phytosterols have become a part of many plant and animal membranes, bearing a polar hydroxyl function at the C (3) carbon centre of the basic skeleton (Vida *et al.*, 2012). They played major roles in several areas, namely in pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anticancer properties) and cosmetic (creams, lipstick) (Fernandes and Cabral, 2007). Apart from that, they may also be implicated in the hypocholesterolemic effect of *R. tuberosa*. Their molecular structure is very similar to that of human cholesterol (Quilez *et al.*, 2003), thus may help in reducing cholesterol absorption by competing with the endogenous cholesterol (Cherki *et al.*, 2006). Besides, they were found to inhibit tumour growth of non-hormone dependent breast cancer (MDA-MB-231) cells (Nachimuthu and Palaniswamy, 2013). These author also reported that stigmasterol induce four to six fold increases in apoptotic death in MDA-MB-231 cells. Stigmasterol also acts as antimicrobial, anticancer, anti-inflammatory, antiasthma, diuretic and antiarthritic (Rejandra *et al.*, 2014). Campesterol, on the other hand, acts as an antinocicpetive (Kamurthy *et al.*, 2013) as well as anticancer (Choi *et al.*, 2007) while gamma-sitosterol acts as an antimicrobial, antiangiogenic and antidiabetic (Venkata *et al.*, 2012). Lupeol, a phytosterol and triterpene, is widely found in edible fruits and vegetables (Siddique and Saleem, 2011) which have gained the medical interest due to its ranging pharmacology activities. As reported by Rajendra *et al.* (2014), lupeol has antimicrobial, anticancer, anti-inflammatory, antioxidant and antiarthritic.

The anti-proliferative activity of Sabah *R. tuberosa* was evaluated by the means of MTT assay. It is one of the common assay used to evaluate the growth/proliferative of living cells (Cheong *et al.*, 2013). Based on the MTT assay (Table 5), the methanol leaf extract showed stronger inhibitory effect against the MCF-7 cell lines with the IC_{50} value of 20 µg/ml. The IC_{50} value refers to 50% growth inhibitory concentration and the lower the IC_{50}, the higher the anti-proliferative activity (Cheong *et al.*, 2013). They have speculated that the anti-proliferative activity of the *R. tuberosa* extracts against the MCF-7 cell line was not only due to the presence of phenolic/flavonoid compounds as reported by Lin *et al.* (2006) and Reddy *et al.* (2013) but may be due to the presence of other classes of compounds as well. Identification of potential anticancer compounds from Sabah *R. tuberosa* was done based on the analysis of the constructed libraries and the MTT assay against MCF-7 cell line as well as by ethnopharmacological knowledge of the plant's phytochemical compounds. The results from the GC-MS along with the MTT assay of *R. tuberosa* plant extracts against MCF-7 cell line allowed us to highly speculated that squalene, stigmasterol, campesterol and lupeol are the potential anticancer compounds for this plant. This is followed by vitamin E which

may also be responsible for the anticancer property of this plant. Further study should be conducted on the isolation of these phytochemical compounds and their anticancer activity against MCF-7 cell line for the development of anticancer drug from *R. tuberosa*.

Table 5 Plant extracts with their phytochemical compounds and peak area

Plant extracts	IC_{50} Value	Phytochemical compounds	% area
Methanol leaf	20 µg/ml	Squalene	17.65
		Vitamin E	14.68
		Stigmasterol	6.09
Ethyl acetate leaf	28 µg/ml	Squalene	74.74
Ethyl acetate stem	31 µg/ml	Squalene	88.19
Hexane leaf	34 µg/ml	Squalene	23.67
		Gamma-tocopherol	1.04
		Vitamin E	17.03
		Campesterol	2.77
		Stigmasterol	5.25
		Gamma-sitosterol	2.87
Hexane root	36 µg/ml	Vitamin E	1.99
		Campesterol	2.18
		Stigmasterol	7.19
		Gamma-sitosterol	2.96
		Lupeol	66.32

CONCLUSION

GC-MS analysis showed the presence of phytochemical compounds with different chemical structures. These phytochemical compounds have various biological activities that could contribute to the therapeutic value of *R. tuberosa*. Among them, squalene, stigmasterol, campesterol, lupeol and vitamin were the prevalent constituents detected in leaf, stem and root of *R. tuberosa*. Based on the PCDL and the MTT assay of *R. tuberosa* against MCF-7 cell line, it is highly speculated that squalene, stigmasterol, campesterol, lupeol and vitamin E were the compounds that hold potential anticancer property of this plant. Further study on the isolation of these compounds and their anticancer activity should be conducted for confirmation.

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